

We claim:

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1. A method of obtaining a selectable transgenic stem cell of a vertebrate, comprising:
administering to a gonad of a male vertebrate a transfection mixture comprising at least one
transfecting agent and at least one polynucleotide comprising a transcriptional unit of a stem cell-
specific promoter operatively linked to a DNA encoding a fluorescent or light-emitting protein,
5 under conditions effective to reach a germ cell or germ cell precursor of the male vertebrate; and
causing said polynucleotide to be taken up by, and released into, said germ cell or precursor
cell;
incorporating said polynucleotide into the genome of said germ cell or precursor cell,
whereby a selectable transgenic stem cell is obtained expressing said fluorescent or light-emitting
10 protein, by which said stem cell can be isolated or selected from a non-stem cell.
2. The method of Claim 1, further comprising, after incorporating said polynucleotide
into the genome of said germ cell or precursor cell, breeding said male vertebrate with a female of
its species to obtain a transgenic progeny expressing said fluorescent or light-emitting protein in at
least one of its stem cells.
3. The method of Claim 2, wherein breeding is by in vitro or in vivo fertilization of an
ovum of said female.
4. The method of Claim 1, wherein said stem cell-specific promoter is a human cyclin
A1 promoter having a nucleotide sequence (SEQ. ID. NO.:2), or an operative fragment or non-human
homologue thereof, or an operative derivative of any of these.
5. The method of Claim 1, wherein said polynucleotide further comprises at least one
insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter
sequence is substantially prevented.
6. The method of Claim 5, wherein at least one of said insulator element(s) is a chicken
 β -globin insulator element.

7. The method of Claim 1, wherein said fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.
8. The method of Claim 1, wherein said vertebrate is a mammal or bird.
9. The method of Claim 1, wherein said vertebrate is a human, non-human primate, mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm or marine mammal.
10. The method of Claim 1, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.
11. The method of Claim 1, wherein said germ cell or precursor cell develops into a maturing male gamete after said polynucleotide is incorporated into the genome of said germ cell or precursor cell.
12. The method of Claim 2, wherein a stem cell of said progeny is grown in vitro.
13. The method of Claim 12, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.
14. A selectable transgenic stem cell obtained by the method of Claim 1.
15. The selectable transgenic stem cell of Claim 14, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.
16. The selectable transgenic stem cell of Claim 14, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

17. The selectable transgenic stem cell of Claim 14, wherein said stem cell is a selectable transgenic male germ cell.

18. A transgenic non-human vertebrate comprising the stem cell of Claim 14.

19. The transgenic non-human vertebrate of Claim 18, wherein said vertebrate is a non-human mammal or a bird.

20. Vertebrate semen comprising a maturing male gamete obtained by the method of Claim 11.

21. A method of producing a non-human transgenic vertebrate animal line having native germ cells, comprising:

breeding the transgenic non-human vertebrate of Claim 18, with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

22. A method of obtaining a selectable transgenic stem cell of a vertebrate, comprising: administering to a gonad of a male vertebrate a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a cyclin A1 promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein, under conditions effective to reach a germ cell or germ cell precursor of the male vertebrate; and causing said polynucleotide to be taken up by, and released into, said germ cell or precursor cell;

incorporating said polynucleotide into the genome of said germ cell or precursor cell, whereby a selectable transgenic stem cell is obtained expressing said fluorescent or light-emitting protein, by which said stem cell can be isolated or selected from a non-stem cell.

23. The method of Claim 22, further comprising, after incorporating said polynucleotide into the genome of said germ cell or precursor cell, breeding said male vertebrate with a female of its species to obtain a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells.

24. The method of Claim 23, wherein breeding is by in vitro or in vivo fertilization of an ovum of said female.
25. The method of Claim 22, wherein said cyclin A1 promoter sequence comprises SEQ. ID. NO.:2, or an operative fragment or non-human homologue thereof, or an operative derivative of any of these.
26. The method of Claim 22, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter sequence is substantially prevented.
27. The method of Claim 26, wherein at least one of said insulator element(s) is a chicken β -globin insulator element.
28. The method of Claim 22, wherein said fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase or apoaquorin.
29. The method of Claim 22, wherein said vertebrate is a mammal or bird.
30. The method of Claim 22, wherein said vertebrate is a human, non-human primate, mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm or marine mammal.
31. The method of Claim 22, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.
32. The method of Claim 22, wherein said germ cell or precursor cell develops into a maturing male gamete after said polynucleotide is incorporated into the genome of said germ cell or precursor cell.
33. The method of Claim 23, wherein a stem cell of said progeny is grown in vitro.

34. The method of Claim 33, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.

35. A selectable transgenic stem cell obtained by the method of Claim 22.

36. The selectable transgenic stem cell of Claim 35, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

37. The selectable transgenic stem cell of Claim 35, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

38. The selectable transgenic stem cell of Claim 35, wherein said stem cell is a selectable transgenic male germ cell.

39. A transgenic non-human vertebrate comprising the stem cell of Claim 35.

40. The transgenic non-human vertebrate of Claim 39, wherein said vertebrate is a non-human mammal or a bird.

41. Vertebrate semen comprising a maturing male gamete obtained by the method of Claim 32.

42. A method of producing a non-human transgenic vertebrate animal line having native germ cells, comprising

breeding of the vertebrate of Claim 39, with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

43. A method of obtaining a selectable transgenic stem cell of a vertebrate, comprising: administering to a gonad of a male vertebrate a transfection mixture comprising at least one transfecting agent and at least one polynucleotide comprising a transcriptional unit of a cyclin A1

promoter sequence operatively linked to a DNA encoding a fluorescent or light-emitting protein,
5 under conditions effective to reach a germ cell or germ cell precursor of the male vertebrate; and
causing said polynucleotide to be taken up by, and released into, said germ cell or precursor
cell;

incorporating said polynucleotide into the genome of said germ cell or precursor cell;

allowing said germ cell or precursor cell to develop into a maturing male gamete; and

10 breeding said male vertebrate with a female of its species to obtain a transgenic progeny
expressing said fluorescent or light-emitting protein in at least one of its stem cells, whereby said
stem cell can be isolated or selected from a non-stem cell.

44. The method of Claim 43, wherein breeding is by in vitro or in vivo fertilization of an
ovum of said female.

45. The method of Claim 43, wherein said cyclin A1 promoter sequence comprises SEQ.
ID. NO.:2, or an operative fragment or non-human homologue thereof, or an operative derivative of
any of these.

46. The method of Claim 43, wherein said polynucleotide further comprises at least one
insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter
sequence is substantially prevented.

47. The method of Claim 46, wherein at least one of said insulator element(s) is a
chicken β -globin insulator element.

48. The method of Claim 43, wherein said fluorescent or light-emitting protein is a green
fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase
or apoaequorin.

49. The method of Claim 43, wherein said vertebrate is a mammal or bird.

50. The method of Claim 43, wherein said vertebrate is a human, non-human primate,
mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm
or marine mammal.

51. The method of Claim 43, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.
52. The method of Claim 43, wherein a stem cell of said transgenic progeny is grown in vitro.
53. The method of Claim 52, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.
54. A selectable transgenic stem cell obtained by the method of Claim 43.
55. The selectable transgenic stem cell of Claim 54, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.
56. The selectable transgenic stem cell of Claim 54, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.
57. The selectable transgenic stem cell of Claim 54, wherein said stem cell is a selectable transgenic female or male germ cell.
58. A transgenic non-human vertebrate comprising the stem cell of Claim 54.
59. The transgenic non-human vertebrate of Claim 58, wherein said vertebrate is a non-human mammal or a bird.
60. A maturing male gamete obtained by the method of Claim 43.
61. Vertebrate semen comprising the maturing male gamete of Claim 60.
62. A method of producing a non-human transgenic vertebrate animal line having native germ cells, comprising

breeding the vertebrate of Claim 58, with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

63. A method of obtaining a selectable stem cell, comprising:

obtaining a maturing male germ cell from a vertebrate;

transfecting said male germ cell in vitro with at least one polynucleotide comprising a transcriptional unit of a stem cell-specific promoter operatively linked to a DNA encoding a fluorescent or light-emitting protein, in the presence of a gene delivery mixture comprising at least one transfecting agent, at about or below the vertebrate's body temperature and for a transfection-effective period of time;

causing said polynucleotide to be taken up by, and released into said germ cell; and fertilizing an ovum with said germ cell such that a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells is obtained, said stem cell(s) being selectable from non-stem cells by detecting light emissions from said stem cell(s).

64. The method of Claim 63, wherein fertilizing an ovum is by in vitro or in vivo fertilization.

65. The method of Claim 63, wherein said stem cell-specific promoter is a cyclin A1 promoter.

66. The method of Claim 63, wherein said cyclin A1 promoter sequence comprises SEQ. ID. NO.:2, or an operative fragment or non-human homologue thereof, or an operative derivative of any of these.

67. The method of Claim 63, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter sequence is substantially prevented.

68. The method of Claim 67, wherein at least one of said insulator element(s) is a chicken β -globin insulator element.

69. The method of Claim 63, wherein said fluorescent protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaeguorin.

70. The method of Claim 63, wherein said vertebrate is a mammal or bird.

71. The method of Claim 63, wherein said vertebrate is a human, non-human primate, mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm or marine mammal.

72. The method of Claim 63, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.

73. The method of Claim 63, wherein a stem cell of said transgenic progeny is grown in vitro.

74. The method of Claim 73, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.

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~~75. A selectable transgenic stem cell obtained by the method of Claim 63,~~

76. The selectable transgenic stem cell of Claim 75, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

77. The selectable transgenic stem cell of Claim 75, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

78. The selectable transgenic stem cell of Claim 75, wherein said stem cell is a selectable transgenic female or male germ cell.

79. A transgenic non-human vertebrate comprising the selectable transgenic stem cell of Claim 75.

80. The transgenic non-human vertebrate of Claim 79, wherein said vertebrate is a non-human mammal or a bird.

81. Vertebrate semen comprising the male germ cell of Claim 78,

82. A method of producing a non-human transgenic vertebrate animal line having native germ cells, comprising

breeding the vertebrate of Claim 79 with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

83. A method of obtaining a selectable stem cell, comprising:
obtaining a maturing male germ cell from a vertebrate;

transfecting said male germ cell in vitro with at least one polynucleotide comprising a transcriptional unit of a cyclin A1 promoter operatively linked to a DNA encoding a fluorescent or light-emitting protein, in the presence of a gene delivery mixture comprising at least one transfecting agent, at about or below the vertebrate's body temperature and for a transfection-effective period of time; and

allowing said polynucleotide to be taken up by, and released into said germ cell;

fertilizing an ovum with said germ cell such that a transgenic progeny expressing said fluorescent or light-emitting protein in at least one of its stem cells is obtained, said stem cell(s) being selectable from non-stem cells by detecting light emissions from said stem cell(s).

84. The method of Claim 83, wherein fertilizing an ovum is by in vitro or in vivo fertilization.

85. The method of Claim 83, wherein said cyclin A1 promoter sequence comprises SEQ. ID. NO.:2, or an operative fragment or non-human homologue thereof, or an operative derivative of any of these.

86. The method of Claim 83, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit, whereby methylation in vivo of said promoter sequence is substantially prevented.

87. The method of Claim 86, wherein at least one of said insulator element(s) is a chicken β -globin insulator element.

88. The method of Claim 83, wherein said fluorescent protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoeaquerin.

89. The method of Claim 83, wherein said vertebrate is a mammal or bird.

90. The method of Claim 83, wherein said vertebrate is a human, non-human primate, mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm or marine mammal.

91. The method of Claim 83, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.

92. The method of Claim 83, wherein a stem cell of said transgenic progeny is grown in vitro.

93. The method of Claim 92, wherein said stem cell is grown in the presence of an inhibitor of DNA methylation.

Sub ³ 94. ~~A selectable transgenic stem cell obtained by the method of Claim 83.~~

95. The selectable transgenic stem cell of Claim 94, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

96. The selectable transgenic stem cell of Claim 94, wherein said stem cell is a spermatogonial, embryonic, osteogenic, hematopoietic, granulopoietic, sympathoadrenal,

mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

97. The selectable transgenic stem cell of Claim 94, wherein said stem cell is a selectable transgenic female or male germ cell.

98. A transgenic non-human vertebrate comprising the stem cell of Claim 94,

99. The transgenic non-human vertebrate of Claim 98, wherein said vertebrate is a non-human mammal or a bird.

100. Vertebrate semen comprising the male germ cell of Claim 97.

101. A method of producing a non-human transgenic vertebrate animal line having native germ cells, comprising

breeding of the vertebrate of Claim 98 with a member of the opposite sex of the same species; and selecting progeny for stem cell-specific expression of a xenogeneic fluorescent or light-emitting protein.

102. A nucleic acid construct, comprising a cyclin A1 promoter having nucleotide sequence (SEQ. ID. NO.:2), or an operative fragment or non-human homologue thereof, or an operative derivative of any of these.

103. The nucleic acid construct of Claim 102, further comprising said cyclin A1 promoter operatively linked to a nucleotide sequence encoding a fluorescent or light-emitting protein, as a transcriptional unit.

104. The nucleic acid construct of Claim 103, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit.

105. The nucleic acid construct of Claim 104, wherein at least one of said insulator element(s) is a chicken β -globin insulator element.

106. The nucleic acid construct of Claim 103, wherein the encoded fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

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107. A transgenic vertebrate cell containing the nucleic acid construct of Claim 102.

108. A transgenic non-human vertebrate comprising the cell of Claim 107.

109. The transgenic non-human vertebrate of Claim 108, wherein said vertebrate is a non-human mammal or a bird.

110. The transgenic vertebrate cell of Claim 107, wherein said cell is a transgenic stem cell.

111. The transgenic stem cell of Claim 110, wherein said stem cell is a pluripotent, multipotent, bipotent, or monopotent stem cell.

112. The transgenic stem cell of Claim 110, wherein said stem cell is a spermatogonial, hematopoietic, embryonic, osteogenic, granulopoietic, sympathoadrenal, mesenchymal, epidermal, neuronal, neural crest, O-2A progenitor, brain, kidney, pancreatic, liver or cardiac stem cell.

113. The transgenic stem cell of Claim 110, grown in vitro.

114. The transgenic stem cell of Claim 113, grown in the presence of an inhibitor of DNA methylation.

115. A transgenic non-human vertebrate comprising the transgenic stem cell of Claim 110.

116. The transgenic non-human vertebrate of Claim 115, wherein said vertebrate is a non-human primate, mouse, rat, rabbit, gerbil, hamster, canine, feline, ovine, bovine, swine, pachyderm, equine, or a farm or marine mammal.

117. The transgenic non-human vertebrate of Claim 115, wherein said vertebrate is a duck, chicken, goose, ostrich, emu, dove, quail, guinea fowl, or turkey.

118. A kit for transfecting a male vertebrate's germ cells, comprising:
a transfecting agent and a polynucleotide comprising a transcriptional unit of a human cyclin A1 promoter sequence having SEQ. ID. NO.:2, or an operative fragment or non-human homologue thereof, or an operative derivative of any of these, operatively linked to a DNA having a nucleotide sequence encoding a fluorescent or light-emitting protein, whereby said kit may be used to transfect said germ cells.

119. The kit of Claim 118, wherein the transfecting agent is a liposome, viral vector, transferrin-polylysine enhanced viral vector, retroviral vector, lentiviral vector, or uptake enhancing DNA segment, or a mixture of any of these.

120. The kit of Claim 118, wherein the transfecting agent comprises a retroviral vector, adenoviral vector, transferrin-polylysine enhanced adenoviral vector, human immunodeficiency virus vector, lentiviral vector, Moloney murine leukemia virus-derived vector, mumps vector, a DNA segment that facilitates polynucleotide uptake by and release into the cytoplasm of germ cells, or comprises an operative fragment of- or mixture of any of these.

121. The kit of Claim 118, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

122. The kit of Claim 118, wherein the transfecting agent comprises a lipid transfecting agent.

123. The kit of Claim 118, wherein the transfecting agent further comprises a male-germ-cell-targeting molecule.

124. The kit of Claim 123, wherein the male-germ-cell-targeting molecule is specific for targeting spermatogonia and comprises a c-kit ligand.

125. The kit of Claim 118, further comprising an immunosuppressing agent.

126. The kit of Claim 125, wherein the immunosuppressing agent is cyclosporin or a corticosteroid.

127. The kit of Claim 123, wherein the kit contains at least one additional polynucleotide comprising a nucleotide sequence encoding for expression of a desired trait.

128. The kit of Claim 127, wherein the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand; and the kit contains at least one additional polynucleotide comprising a nucleotide sequence encoding for expression of a desired trait.

129. The kit of Claim 123, wherein the male-germ-cell-targeting molecule is specifically targeted to spermatogonia and comprises a c-kit ligand; and

the DNA having a nucleotide sequence encoding a fluorescent protein is operatively linked to a cyclin A1 promoter, c-kit promoter, B-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, or FRMI (from fragile X site) promoter.

130. The kit of Claim 118, wherein said polynucleotide further comprises at least one insulator element flanking said transcriptional unit.

131. The kit of Claim 130, wherein at least one of said insulator element(s) is a chicken β -globin insulator element.

132. The kit of Claim 118, wherein said fluorescent or light-emitting protein is a green fluorescent protein, yellow fluorescent protein, blue fluorescent protein, phycobiliprotein, luciferase, or apoaequorin.

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